for the apoptotic morphology changes. Sanguinarine-mediated apoptosis was associated with the increase of nitric oxide (NO) formation in prostate cancer cells as assessed by measurements of nitrites with Sievers Nitric Oxide Analyzer as well as flow cytometry analysis using NO fluorescent sensor. Activation of Nitric Oxide Synthase (NOS) activity was crucial for apoptotic effect of sanguinarine, since NOS inhibitor L-NMMA efficiently protected cells from apoptosis. In order to test whether elevated Cox-2 expression contributes to prostate cancer cell resistance to anti-cancer drugs, we used adenovirus-mediated transfer of gene encoding Cox-2 into LNCaP and PC3 cells. Enforced Cox-2 expression inhibited sanguinarine-induced apoptosis and prevented increase in NO production. Surprisingly, NO donors failed to induce apoptosis in LNCaP cells, suggesting that constitutive NO generation is not sufficient for triggering apoptosis in these cells. Besides NO generation, NOS is capable to produce superoxide radicals. Addition of a membrane-permeable scavenger of superoxide radicals MnTBAP efficiently inhibited sanguinarine-mediated apoptosis. The ability of Cox-2 to prevent sanguinarine-mediated formation of reactive oxygen species is currently under investigation.

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EXPRESSION OF NOX1 AND NOX4 IN NEURONAL APOPTOSIS

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The generation of reactive oxygen species (ROS) is necessary to trigger apoptosis in many models of neurodegeneration. Recently, this burst of ROS has been linked to the activation of NADPH oxidase(s) in NGF-deprived sympathetic neurons, however the identity of the specific NADPH oxidase subunits has not been established. In the present study, the expression patterns of Nox1 and Nox4, two homologs of the phagocytic NADPH oxidase subunit gp91-phox, were investigated in NGF-deprived sympathetic neurons and differentiated PC12 cells. Semi-quantitative RT-PCR, quantitative Real-time PCR, and western blot analysis revealed that Nox4 is up-regulated within three hours after NGF withdrawal from sympathetic neurons and PC12 cells, followed by a down-regulation at the mRNA and protein levels by six hours after NGF deprivation. Double immunofluorescence visualized with confocal laser scanning microscopy indicated the co-localization of both Nox1 and Nox4 with the membrane-bound p22-phox subunit. These results suggest that Nox4, and perhaps Nox1, may be the functional NADPH oxidase subunits that generate ROS following NGF withdrawal in sympathetic neurons and differentiated PC12 cells. Here, we report the expression patterns, partial sequence and distribution of Nox1 and Nox4 in these two cell types.

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LEPORIPOXVIRUSES SOD1 HOMOLOGS UP-REGULATE INTRACELLULAR SUPEROXIDE LEVEL WHICH PROMOTES TUMORIGENESIS AND PREVENTS APOPTOSIS

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The accumulation of intracellular ROS has been suggested to provide tumor cells with a survival advantage since aberrant SOD1/2 activities are often reported in those cells. Interestingly, tumorigenic Leporipoxviruses encode catalytically-inactive homologs of cellular SOD1 (S/M131R). These virus proteins interact with cellular copper chaperone for SOD1, thereby inhibiting the cellular SOD1 activity. As a consequence, the expression of S/M131R is accompanied by an increase in intracellular superoxide level as indicated by confocal microscopy of hydroethidine (HE) staining. To examine the role of Shope fibroma virus SOD1 homolog in mediating virus tumorigenesis, a mutant virus encoding a S131R gene deletion was generated and the clinical manifestations in rabbits were compared with infections caused by the wild-type virus. Deleting S131R significantly reduced the size of tumors formed at the site of inoculation, and they regressed much faster than those caused by wild-type virus. Histologically, there were extensive areas of leukocyte infiltration, necrosis, and fibromatous cell proliferation in wild-type virus group at day 13, whereas tissues infected with mutant viruses were already showing signs of healing. Since superoxide has long been implied in regulating apoptosis and we have shown that the S/M131R-induced pro-oxidant state protects infected human Jurkat T-cells from both mitochondrial and Fas-mediated apoptosis. Both DNA laddering and TUNEL assay indicate that M131R deletion mutant virus has a pro-apoptotic phenotype. Collectively, these data suggest that tumorigenic poxviruses use SOD1 homologs to modulate intracellular redox status, which could be of advantages to the viruses in stimulating cell growth and interfering with programmed cell death.

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TOLERANCE OF INCREASED ROS PROTECTS AGAINST GLUCOCORTICOID-INDUCED APOPTOSIS IN LYMPHOID CELLS.

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Glucocorticoids induce apoptosis in lymphoid cells via the mitochondrial stress pathway where release of cytochrome c into the cytosol is the committed step. An increase in ROS prior to cytochrome c release has been implicated in the mechanism of steroid-induced apoptosis. Previously, we found that WEHI7.2 murine thymic lymphoma cells that overexpress thioredoxin or catalase by transfection or are selected for resistance to hydrogen peroxide are all resistant to glucocorticoid-induced apoptosis. The oxidative stress-resistant WEHI7.2 variants show a delay in or lack of cytochrome c release indicating the protection occurs during apoptotic signaling in these variants. The protection could be due to either more efficient removal or increased tolerance of ROS. To distinguish between these two possibilities, we compared ROS after steroid treatment in the variants to that in the parental cells. The parental cells and all the variants showed an increase in ROS and glutathione S-transferase due to glucocorticoid treatment suggesting that steroids cause oxidative stress in all the cells. Cardiolipin oxidation, which may play a role in cytochrome c release, occurs in the parental cells and some, but not all, of the variants. Cardiolipin oxidation was not correlated to sensitivity to steroid-induced apoptosis. These data suggest that tolerance of increased ROS plays a role in the glucocorticoid resistance of the WEHI7.2 variants.

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CD95-INDUCED CELL DEATH IS MEDIATED BY REACTIVE OXYGEN SPECIES

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Although reactive oxygen species (ROS) have long been suspected to play a key role in Fas (CD95)-induced cell death, the identity of specific ROS involved in this process and the relationship between apoptotic and necrotic cell death induced by Fas are largely unknown. Using electron spin resonance (ESR) spectroscopy, we showed that activation of Fas receptor by its ligand (FasL) in macrophages resulted in a rapid and transient production of hydrogen peroxide (H2O2) and hydroxyl radicals (.OH). The response was visible as early as 5 min and peaked at

approximately 45 min post treatment with FasL. Morphological analysis of total death response showed a significant increase in apoptosis at 6 h after the treatment, while necrosis remained at a baseline level. Only at a 35-fold increase in apoptosis did necrosis become significant. Inhibition of apoptosis by a pan-caspase inhibitor, zVAD-fmk, significantly inhibited cell necrosis, indicating the linkage between the two events. Catalase (H2O2 scavenger) and deferoxamine effectively inhibited the total death response as well as the ESR signals, while superoxide dismutase (SOD) (O2.- scavenger) had minimal effects. These results established the role for H2O2 and .OH as key participants in Fas-induced cell death and indicated apoptosis as a primary mode of cell death preceding necrosis. Because the Fas death pathway is implicated in various inflammatory and immunologic disorders, utilization of antioxidants and apoptosis inhibitors as potential therapeutic agents may be advantageous.

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DECREASED SUPEROXIDE AND HYDROGEN PEROXIDE LEVELS INDUCE GROWTH ARREST AND APOPTOSIS IN ENDOTHELIAL CELLS

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Reactive oxygen species such as superoxide and hydrogen peroxide are known to play an important role in the proliferation and viability of vascular smooth muscle cells. Increasing data suggest that anti-oxidant therapy may be of benefit in the treatment of cardiovascular diseases resulting from excessive muscularization. However, little is known about the effects of anti-oxidant treatment on the endothelium. In this study we monitored the growth and viability of endothelial cells (ECs) isolated from the pulmonary arteries of fetal lambs in response to EUK-8, a superoxide dismutase/catalase mimetic. EUK-8 dose-dependently decreased superoxide and hydrogen peroxide levels in ECs as detected by the fluorescent dyes dihydroethidium and dichlorodihydrofluorescein diacetate. At low doses (5μM) EUK-8 attenuated serum-induced EC proliferation while high doses (500μM) significantly decreased the number of viable cells relative to the number initially seeded. Furthermore, high doses of EUK-8 decreased mitochondrial membrane potential, increased levels of active caspase-3 and induced DNA fragmentation, indicating apoptosis. These data suggest that superoxide and hydrogen peroxide levels regulate EC growth, and that their removal can initiate programmed cell death. Thus, the use of anti-oxidants to treat over-muscularized vessels must be closely monitored to avoid impairment of normal endothelial function.

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EXPRESSION OF PROLIFERATION MARKERS CYCLIN D1 AND KI67 IN MOUSE LUNG AFTER EXPOSURE TO ASBESTOS AND NITROGEN DIOXIDE

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Exposure of mice to nitrogen dioxide (NO2) or crocidolite asbestos results in pulmonary injury and induction of compensatory epithelial cell proliferation by 3-4 days. The aim of our study was to evaluate distribution and changes in expression of Ki67 and cyclin D1, two proteins expressed primarily in proliferating cells, in lung tissue after injury. Frozen lung sections from mice exposed to asbestos, NO2, or both, and an unexposed control were immunostained for Ki67 and cyclin D1, with Sytox green nuclear stain. In unexposed tissue, very low levels of cytoplasmic cyclin D1 are detected, as expected for nonproliferating cells. Ki67 and cyclin D1 expression increased markedly in airway epithelium, but not in parenchyma, after all exposures. Exposure to NO2 alone produced the largest increases in nuclear Ki67 expression, followed by asbestos exposure. Cyclin D1 and Ki67 co-expression was observed infrequently. To address the role of these factors in cell proliferation, we adapted a chromatin extraction procedure to frozen lung sections and examined the stable association of proteins with nuclear chromatin. Stable association of cyclin D1 with chromatin has been reported in actively cycling cells. After chromatin extraction, immunostaining revealed a very small subset of cells with chromatin-bound Ki67 or cyclin D1. In cultured mouse type II alveolar cells, Ki67 was expressed immediately after cell cycle reentry, but disappeared rapidly in response to reactive nitrogen species exposure. Experiments to assess the relationship between expression and chromatin binding of cyclin D1 and Ki67 and subsequent S phase entry will be reported. While cyclin D1 and Ki67 are widely used as proliferation markers, our studies indicate that these proteins display distinct expression patterns, suggesting they may identify different cellular responses to injury.

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HOCL-MEDIATED BAX-DEPENDENT MITOCHONDRIAL PERMEABILITY TRANSITION AND CASPASE INDEPENDENT APOPTOTIC CELL DEATH; INHIBITION WITH SMALL INTERFERING RNA TECHNOLOGY

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In the inflamed rheumatoid joint, articular chondrocytes (HAC) are exposed to high μM concentrations of the neutrophil oxidant hypochlorous acid (HOCl). Although the effects of HOCl on the extracellular matrix components of the human joint are well documented, the effects of HOCl on HAC are unknown. Thus, we have used small interfering RNA (siRNA) technology, electron and confocal microscopy and flow cytometry to investigate the effects of HOCl on HAC and mitochondria *in situ*. HOCl induced Bax N-terminal exposure and mitochondrial insertion resulting in mitochondrial permeability transition (MPT) and release of pro-apoptotic mitochondrial proteins AIF, EndoG and SMAC/Diablo. Cell death involved phosphatidylserine externalisation, formation of subG1 cell populations, chromatin condensation and cell body shrinkage, processes highly suggestive of apoptosis. However in HAC, HOCl inhibited caspase-3 and -9 activation. HOCl-induced MPT, release of pro-apoptotic mitochondrial proteins and cell death were substantially inhibited with siRNA Bax knockdown and less so with Ku70 peptide. These data are the first demonstration of HOCl-mediated mitochondrial dysfunction, Bax 'activation' and caspase-independent cell death through AIF / EndoG. The possible redox modulation of Bax and potential for therapeutic interventions in inflammatory joint diseases is discussed.

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MITOCHONDRIAL PERMEABILITY TRANSITION AND APOPTOTIC CELL DEATH MEDIATED BY THE NEUTROPHIL-DERIVED REACTIVE CHLORINE SPECIES HYPOCHLOROUS ACID

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Liver cirrhosis is often preceded by overt signs of hepatitis including parenchymal cell inflammation and infiltration of polymorphonuclear (PMN) leukocytes. Activated PMNs release both reactive oxygen species (ROS) and reactive halogen species (RHS) including hypochlorous acid (HOCl) which are known to be significantly cytotoxic due to their oxidizing potential. Since the role of mitochondria in the hepatotoxicity attributed to HOCl has

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